EXHIBIT 6

CANCER ORIGINS — OPINION

The disparate origins of ovarian cancers: pathogenesis and prevention strategies

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Abstract | Ovarian cancer is the fifth cause of cancer-related death in women and comprises a histologically and genetically broad range of tumours, including those of epithelial, sex cord-stromal and germ cell origin. Recent evidence indicates that high-grade serous ovarian carcinoma, clear cell carcinoma and endometrioid carcinoma primarily arise from tissues that are not normally present in the ovary. These histogenetic pathways are informing risk-reduction strategies for the prevention of ovarian and ovary-associated cancers and have highlighted the importance of the seemingly unique ovarian microenvironment.

Historically, one of the main reasons why the biology and evolution of common ovarian cancers have been so difficult to understand is because most tumour cells do not phenotypically resemble any normal cells in the ovary. For high-grade serous carcinoma (HGSC), the most common ovarian cancer, no credible histological precursor lesion had been identified until 15 years ago, and the majority of mucinous carcinomas of the ovary are metastases from other organs[1]. For other ovarian tumours, for which a precursor lesion has been identified, such as endometriosis for clear cell carcinoma (CCC) and endometrioid carcinoma (EC), it is still not known how the precursor develops[2]. These and other issues have substantially hampered our understanding of the origin of ovarian cancers and their pathogenesis, and thereby limited our ability to study them in experimental systems. Furthermore, the realization that the most common ovarian cancer types arise from cells that are not normally located in the ovary challenges the concept of what a 'true' ovarian cancer is.

In this Opinion article we discuss the origins of the three most common and some illustrative, rare ovarian cancers (TABLE 1). Although some cancers arise from cells that exist in histologically normal ovaries, most cancers are derived from cells that typically reside in extra-ovarian tissue. We focus on pathogenic mechanisms and current knowledge gaps and on how these contribute to our understanding of genetic and environmental risk and protective factors, as well as on emerging prevention opportunities.

The real intra-ovarian cancers

Several lines of evidence can be used to indicate whether the ovary is the primary site of origin of an ovarian cancer. For example, the presence of a solitary intra-ovarian tumour (anatomical evidence), histological and immunophenotypic similarities of a tumour to a normal ovarian cell type (phenotypic evidence) and the ability to mutate a normal ovarian cell type in an experimental system and recapitulate the hallmarks of cancer[3] relevant to a particular tumour (biological evidence) can all be used to support evidence that a particular tumour has arisen from cells that are present in a normal ovary. Furthermore, the existence of similar tumours in the ovary and testis suggests an intra-gonadal origin from the analogous cells present in each gonad (circumstantial evidence).

There are tumours that clearly arise from cells native to the ovary, such as germ cell tumours, which have been comprehensively reviewed by Cools *et al.*[4], and are not discussed further in this Opinion article. We focus instead on two gonadal stromal tumours — adult granulosa cell tumour (AGCT) and Sertoli–Leydig cell tumour (SLCT) — in which recent progress has been made in understanding their pathogenesis.

SLCTs and AGCTs are conceptually opposite sides of the same coin: tumours of male or female sex cord cells, respectively, that express transcription factors with antagonistic roles in sex determination (SOX9 and forkhead box protein L2 (FOXL2), respectively). Embryonic gonads are initially bipotent and then develop into either testes or ovaries. In the absence of the male differentiation programme (which is normally induced by the expression of sex-determining region Y protein (SRY) and SOX9 transcription factors[5]), the embryonic gonad develops into ovarian tissue through a programme that is coordinated by sex hormones, FOXO and FOXL2 transcription factors, and the transforming growth factor-β (TGFβ)–SMAD, WNT–β-catenin and RAS–MAPK signalling pathways[6]. Once formed, the predominant cells in the cortex are ovarian stromal cells and ovarian follicles, which consist of oocytes (which have migrated to the ovary from the yolk sac) and the surrounding granulosa and theca cells (FIG. 1). The cortex is covered by a specialized coelomic epithelium that is known as the ovarian surface epithelium (OSE).

Adult granulosa cell tumour. AGCTs represent approximately 5% of ovarian cancers. All evidence points to the granulosa cell as the cell of origin: most AGCTs are confined to the ovary (anatomical evidence) and show histological, immunophenotypic[7–10] and endocrinological similarities[11,12] to normal granulosa cells (phenotypic evidence), and the tumours can rarely occur in the testis[13,14] (circumstantial evidence). However, the broad age range (18–83 years[15]) over which this tumour is diagnosed challenges this hypothesis, as granulosa cells are absent after menopause. However, AGCTs are

Table 1 | **Ovarian tumour origins, risk factors and prevention strategies**

Cancer type	Possible histogenesis (tissue of origin)	Possible cells of origin	Precursor lesion	Familial risk‡	Prevention strategies
HGSC	Fallopian tube fimbria or ovarian cortical inclusion cysts	Fallopian tube secretory epithelial cell or progenitor cell	STIC	*BRCA1*, *BRCA2*, *BRIP1*, *PALB2*, *RAD51C* and *RAD51D*	RRSO, opportunistic salpingectomy, oral contraceptives or tubal ligation
EC, CCC	Endometriosis or endometrioid adenofibroma	Endometrial epithelial cell	Endometrioid borderline tumour	Lynch syndrome (*MLH1*, *PMS2*, *MSH2* and *MSH6*)	Tubal ligation, opportunistic salpingectomy or oral contraceptives
LGSC	Endosalpingiosis or papillary tubal hyperplasia	Fallopian tube secretory epithelial cell or progenitor cell	Serous borderline tumour	NA	NA
Mucinous carcinoma	Unknown; teratoma, endometriosis or tubal–peritoneal junction?	Unknown; normal ovarian cell, somatic cell within a teratoma or endometriosis (for seromucinous tumours)?	Mucinous borderline tumour, Brenner tumour, teratoma or endometriosis	NA	Tubal ligation or oral contraceptives
AGCT	Ovarian follicle	Granulosa cell	None	NA	NA
SLCT	Unknown	Granulosa cell or other stromal cell	None	DICER1 syndrome	NA
Fibroma	Ovarian stromal cell	Ovarian stromal cell	None	None	NA
SCCOHT	Unknown; teratoma	Unknown; normal ovarian cell or somatic cell within immature teratoma	None	RTPS2 (*SMARCA4*)	None

AGCT, adult granulosa cell tumour; *BRIP1*, BRCA1-interacting C-terminal helicase 1; CCC, clear cell carcinoma; EC, endometrioid carcinoma; HGSC, high-grade serous carcinoma; LGSC, low-grade serous carcinoma; *MLH1*, mutL homologue 1; NA, not applicable; *PALB2*, partner and localizer of BRCA2; RAD51C, RAD51 paralogue C; RRSO, risk-reducing salpingo-oophorectomy; RTPS2, rhabdoid tumour predisposition syndrome type 2; SCCOHT, small cell carcinoma of the ovary, hypercalcaemic type; SLCT, Sertoli–Leydig cell tumour; STIC, serous tubal intraepithelial carcinoma. ‡High- and moderate-risk susceptibility genes.

generally slow-growing tumours with a propensity for late recurrence, so it is plausible that some tumours initiate before menopause and grow slowly, presenting after menopause.

Almost all AGCTs have an identical pathognomonic missense mutation (c.402C>G, p.C134W) in the *FOXL2* gene[16–18] (this mutation changes a highly conserved cysteine residue to a tryptophan). The near-universal presence of this mutation in AGCTs and its absence in other cancer types suggests that AGCT is a histomolecular entity[19]. However, FOXL2 is expressed in other stromal cells in the ovary and throughout the Müllerian tract in mice[20] and humans (A.N.K., C.B.G. and D.G.H., unpublished observations), so the biological basis of the specificity of the *FOXL2* mutation for AGCT as opposed to other stromal tumours is unclear. The precise mechanism through which the *FOXL2* missense mutation is oncogenic is not known. The existence of AGCT in postmenopausal women suggests that one potential function of mutant FOXL2 protein is to prevent apoptosis in response to signals that induce the physiological regression of granulosa cells[21,22], potentially through deregulation of the TGFβ signalling pathway, which regulates normal granulosa cell biology and induces granulosa cell tumours in mice[23–25].

FOXL2 is one of the earliest markers expressed during ovarian development[26] (FIG. 2). Inactivating mutations in human *FOXL2*, different from the C402G mutation in AGCTs, result in autosomal dominant blepharophimosis–ptosis–epicanthus inversus syndrome (BPES)[27] type I (Mendelian Inheritance in Man (MIM): 605597; Online Mendelian Inheritance in Man (OMIM): 110100), with granulosa cell dysfunction and premature ovarian failure; mice lacking *Foxl2* show a similar phenotype[26,28]. *Foxl2* deletion in adult mouse granulosa cells leads to expression of the pro-male transcription factor SOX9 and transdifferentiation of female granulosa and theca cells to male Sertoli and Leydig cells[29]. Thus, FOXL2 normally promotes and maintains granulosa cell differentiation and survival for correct ovarian function. AGCTs are rare in the testis compared with the ovary, and testicular AGCTs rarely have a *FOXL2* mutation[13,14]. This may reflect the paucity of FOXL2-expressing cells in the adult testis[30] as substrates for mutation and the inefficiency with which *FOXL2* mutations induce a reversal phenotype from Sertoli cells to granulosa cells.

Sertoli–Leydig cell tumour. SLCTs are rare tumours that arise in young to middle-aged women and often present with androgenic symptoms. The rarity of this cancer might reflect its probable pathway of development, which involves mutations promoting Sertoli cell generation (that is, male differentiation) from granulosa cells.

The discovery of *DICER1* mutations in sporadic and inherited SLCTs[31] was a breakthrough in understanding SLCT biology. The study of *DICER1* mutations in this tumour has offered a variant of Knudson's two-hit hypothesis, as one allele of *DICER1* is truncated in the germ line and a second specific hypomorphic missense mutation is present in tumour tissue[31,32]. This combination of mutations does not completely inactivate DICER1 but instead skews microRNA (miRNA) processing towards 3′ strands[32,33]. How this is oncogenic and how it leads to a tumour that reflects a very unusual cell fate decision is not known. On the basis of the active antagonism between male (SRY and SOX9) and female (WNT and FOXL2) differentiation signals in the development and maintenance of male and female gonadal states[5,29,34], it seems to be reasonable that miRNA-processing abnormalities that are induced by *DICER1* mutations disrupt the balance between

these sex-determining signals to cause a male phenotypic switch. SLCTs in males rarely have *DICER1* mutations, supporting the evidence that *DICER1* mutations contribute to the formation of SLCTs in women by inducing a SOX9 transcriptional programme[35,36].

Ovarian tumours of unknown origin

Small cell carcinoma of the ovary, hypercalcaemic type. Small cell carcinoma of the ovary, hypercalcaemic type (SCCOHT) is perhaps the most lethal and least understood ovarian cancer. It affects young women and children (average age 24 years), and the risk of development is inherited in some cases[37–39]. Histologically, the tumours grow as sheets and poorly formed nests of small cells with scant cytoplasm, hyperchromatic nuclei and prominent nucleoli. Approximately half of these tumours contain larger cells, some of which have rhabdoid features (that is, features suggestive of rhabdomyoblastic differentiation, such as eccentric nucleus, prominent nucleolus and abundant eosinophilic cytoplasm). The cell of origin is not known, because neither the tumour cell histology nor the immunophenotype resembles any normal cell in the ovary (or in the rest of the body). Almost all tumours are diploid[38], and, until recently, no recurrent mutations had been identified.

In 2014, frequent inactivating somatic or germline mutations in *SMARCA4* were identified in SCCOHT[40–43]. SMARCA4 (also known as BRG1) is one of two core ATPases of the SWI/SNF chromatin remodelling complex. SCCOHTs show histological and genetic similarities to malignant rhabdoid tumours of the brain and other organs, which are caused by somatic or germline mutations in *SMARCB1*[44], another core component of the SWI/SNF complex. These observations have led to a proposal that SCCOHT is essentially a rhabdoid tumour of the ovary[45], and it has now been included in rhabdoid tumour predisposition syndrome type 2 (RTPS2, MIM: 603254; OMIM: 613325). We would favour a change in the nomenclature of SCCOHT to a term that incorporates both morphology and genetics, for instance, 'SMARCA4-deficient undifferentiated carcinoma of the ovary', as proposed by Agaimy[46].

In addition to SMARCA4 loss, SCCOHTs lack the other SWI/SNF complex ATPase, SMARCA2 (also known as BRM)[47,48], a combination that is usually lethal[49,50]. Whereas SMARCA4 loss is genetic, no *SMARCA2* mutations have been identified in any SCCOHT. SMARCA2 is expressed in essentially all cells in the ovary[48], therefore the lack of SMARCA2 is probably attributable to epigenetic silencing[51,52], RNA degradation[53] or to the tumour arising from an unusual SMARCA2-negative cell type (FIG. 3).

Understanding the cell of origin of SCCOHT will be essential for developing rational prevention strategies in patients with germline *SMARCA4* mutations. Current evidence indicates that germline mutations show incomplete penetrance for the development of SCCOHT[43] (that is, not all individuals who inherit germline *SMARCA4* mutations develop SCCOHT), which complicates genetic counselling. The wide age range at presentation (14 months to 47 years[38,54]) does not offer an obvious time window for prophylactic oophorectomy (removal of the ovary). The first prophylactic bilateral oophorectomy did not reveal histological or immunohistochemical evidence of SMARCA4-negative candidate precursor cells in the ovary[55]. Intriguingly, Kupryjanczyk and colleagues[41] identified microscopic foci of immature teratoma in two SCCOHT samples, one of which also had a focus of a yolk sac tumour. This finding suggests that SCCOHT can arise from a subpopulation of cells (for example, immature neuroepithelium or yolk sac tumour) in a teratoma (FIG. 3).

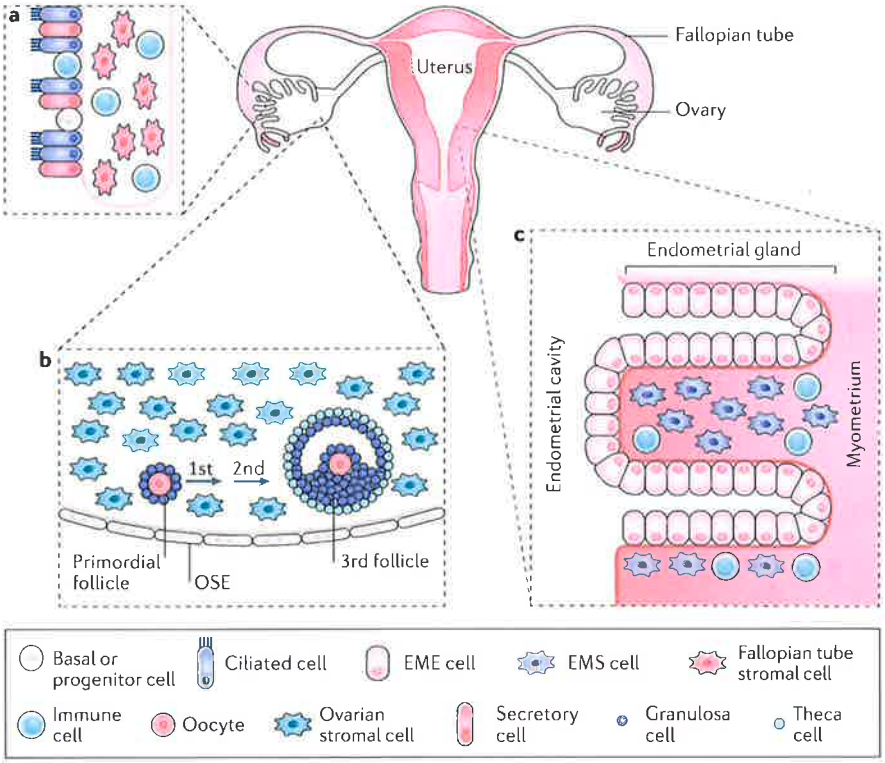

Figure 1 | **Anatomy of the ovary, fallopian tube and uterus.** The insets depict the principal functional cells relevant to organ physiology and ovarian tumorigenesis. **a** | The fallopian tube epithelium contains two principal types of epithelial cell — secretory cells and ciliated cells — that are responsible for fluid synthesis and egg transport. Egg transport is facilitated by contractions of the muscular wall of the tube (not shown). The small basally located cells denote recently characterized epithelial stem and/or progenitor cells[127,128]. Immune cells, which are present in the epithelium and stroma[157], are thought to play a part in immune surveillance. **b** | The ovary inset depicts the principal cells of the ovarian cortex — ovarian surface epithelium (OSE), ovarian stromal cells and cells of ovarian follicles at various stages of maturation, such as oocytes, granulosa cells and theca cells. Granulosa cells and theca cells synthesize oestrogens and androgens, respectively, and support oocyte development. The OSE is involved in the repair of the ovarian surface after ovulation. **c** | The endometrium is composed of cells of the endometrial epithelium (EME) and endometrial stroma (EMS), which respond to systemic and paracrine signals and undergo cyclical changes during the menstrual cycle. During fertilization and implantation, these cells support the nourishment of the embryo. These two cell types — EME and EMS — are present in ectopic endometrial tissue, that is, endometriosis. Whereas endometrial physiology has been studied extensively, the biology of the fallopian tube epithelium and stroma is poorly understood.

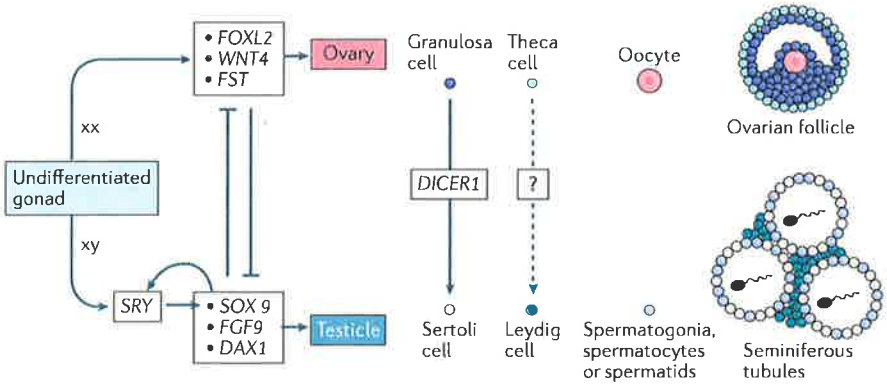

Figure 2 | **Adult granulosa cell tumour and Sertoli–Leydig cell tumour — a developmental perspective.** The female and male gonads develop from a common undifferentiated or indifferent gonad that is induced to undergo differentiation towards the female or male state, resulting in the development of ovarian follicles (see also FIG. 1) or seminiferous tubules, respectively. The genes and pathways that control gonadal sex determination include forkhead box protein L2 (*FOXL2*), *WNT4* and follistatin (*FST*) for ovary determination and sex-determining region Y protein (*SRY*), *SOX9*, fibroblast growth factor 9 (*FGF9*) and the nuclear receptor *DAX1* for testis determination. They are self-reinforcing and cross-inhibitory during gonadal development and in the adult gonads[29,158]. A mutation in *FOXL2* in granulosa cells is likely to enhance its normal role in promoting granulosa cell specification, proliferation and survival[22,26,28], resulting in a granulosa cell tumour. Mutations in *DICER1* in a normal ovarian cell, such as a granulosa cell, may alter the gene expression programme to inhibit the pathways that normally suppress the testicular differentiation programme to alter the cell fate of an ovarian cell towards a male phenotype, resulting in an ovarian Sertoli–Leydig cell tumour.

If many SCCOHTs arise from teratomas or malignant germ cell tumours, screening by imaging or detection of circulating tumour markers (for example, α-fetoprotein for yolk sac tumour) could provide an opportunity for prophylactic removal of the precursor tumour. SCCOHTs also display a curious clinical behaviour, as they only seem to affect one ovary, even in advanced-stage disease[38]. This raises the question of whether both ovaries are at risk in women with germline *SMARCA4* mutations, which could potentially influence treatment and prevention strategies. A mouse model is urgently needed to better understand and develop treatments for this cancer.

Cancers of non-ovarian origin

The most common ovarian carcinomas are thought to arise from cells that are not normally present in the ovary. However, these cells are linked with ovary development. Although anatomically and functionally distinct, both the fallopian tubes and the uterine corpus are lined by specialized epithelium and stroma and are regulated by ovarian sex hormones. The two principal cells in the fallopian tube epithelium (FTE) are secretory and ciliated cells (FIG. 1). In the uterine corpus, the endometrial epithelium and underlying stromal cells line the uterine cavity. The coordination of the menstrual cycle by the hypothalamic–pituitary–ovarian axis is well studied. However, the fallopian tube also undergoes an evolutionarily conserved cycle that is regulated by oestrogen and progesterone[56–59]. The mechanisms through which the ovary regulates the biology of the FTE and the implications for the pathogenesis and prevention of ovarian cancers are not well understood[60]. Despite this, cells from both the uterine cavity and the fallopian tube are implicated in the development of several ovarian cancers, such as EC, CCC and HGSC, as discussed below. Clinicopathological, molecular and animal studies support this hypothesis, and recent studies indicate new cancer prevention strategies[61,62].

Endometriosis-associated cancers

Proposed origins of endometriosis. Endometriosis, defined as endometrial tissue outside the uterine cavity, affects up to 10% of the female population[63]. It is commonly found on the ovaries (where it can develop into blood-filled endometriotic cysts; also known as endometriomas) and on other pelvic or abdominal structures. Endometriosis has been proposed to develop by retrograde or vascular dissemination during menstruation, through metaplasia or from Müllerian developmental remnants including stem-like cells[2].

Although endometriosis had long been considered a non-neoplastic biphasic growth featuring endometrial glandular epithelium and specialized endometrial stroma, it is also an established risk factor for CCC and EC[64]. Genomic analysis of these cancers, which have been described as endometrial cancers in the wrong place[65], has provided new insights into the nature of endometriosis associated with carcinomas[66–68].

Endometriosis as a precursor of ovarian cancer. The coexistence of endometriosis and ovarian cancer is well established[69]. Atypical endometriosis is an intermediate precursor that links conventional endometriosis and ovarian cancers on the basis of its temporal precedence[70] (that is, endometriosis occurring before ovarian cancer), direct histological continuity with cancer[71,72] and significantly higher prevalence in endometriosis associated with cancer (herein referred to as 'high-risk endometriosis') than in endometriosis not associated with cancer (herein referred to as 'low-risk endometriosis')[72–74]. Loss-of-heterozygosity studies have demonstrated clonal relationships between endometriosis and adjacent cancers[75,76]. Mutational studies have shown similar mutations in *PTEN* and AT-rich interaction domain 1A (*ARID1A*) in cancers and adjacent endometriosis[66–68,77].

More recently, whole-genome sequencing has demonstrated high mutational burden and clonal relationships between tumours and both adjacent and spatially distinct endometriosis[66]. The clonal relationship between multiple physically separate endometriosis lesions suggests that these lesions are derived from a common, mobile precursor that continues to evolve genetically at each site of implantation. The clonal relationship of synchronous endometrial and ovarian ECs supports this notion[78]. In light of the strong protective effect of bilateral tubal ligation against endometriosis-associated cancers[61,79], these data are most consistent with retrograde menstruation as the main mechanism underlying cancer-associated endometriosis.

Biological correlates of high-risk endometriosis. The biphasic histology of endometriosis and the development of diverse cancers from ovarian endometriotic cysts (blood-filled cysts) suggest that the pathogenesis of endometriosis-associated cancers involves a complex interplay between genetic alterations in endometriotic epithelium[66,67,77,80], epigenetic alterations in endometriotic stroma[68,81] and the nature of the microenvironment, including the surrounding ovary, immune infiltrates[82] and the mutagenic nature of the blood-filled cyst[83]. Although the epidemiological,

anatomical and genetic relationships between the epithelium of ovarian endometriosis and ovarian carcinomas are irrefutable, many gaps in our knowledge preclude the development of effective strategies to prevent such cancers. Many endometriotic lesions occur outside the ovary, but carcinomas at such sites are rare. The ovarian microenvironment seems to be crucial for neoplastic transformation of endometriosis (discussed below). Understanding the time frame in which mutations and epigenetic events that drive transformation occur could help to develop tools to predict which women who develop recurrent endometriomas[84] might have a residual cancer risk. Many questions remain: in particular, is it possible to determine a priori whether an endometrioma has the potential to transform into cancer, and how do two very different cancers — CCC and EC — arise from the same precursor lesion?

High-grade serous carcinoma

High-grade serous carcinomas originating in the fallopian tube. HGSC often starts in the fallopian tube as serous tubal intraepithelial carcinoma (STIC). Insights over the past 15 years into the tubal origin of ovarian HGSC have resulted in a seismic shift in our understanding of the pathogenesis and prevention of the most deadly adult ovarian cancer[85–87]. The previously favoured 'incessant-ovulation hypothesis' (REF. 88) proposed that repeated rupture and repair of the ovarian surface led to increased proliferation, metaplasia of the OSE to a Müllerian phenotype and accumulation of deleterious mutations that resulted in HGSC. Although the introduction of Simian virus 40 large T antigen (SV40 Tag) into the OSE can induce aggressive carcinomas in mice[89–92], the rarity of credible histological precursor lesions on, or in, women's ovaries[93] was attributed to their destruction during tumorigenesis. In 1999, Dubeau[94] suggested that the Müllerian epithelium, rather than the OSE, could be the source of these cancers. It was not until the introduction of risk-reducing salpingo-oophorectomy (RRSO) in women with germline mutations in *BRCA1* and *BRCA2* that credible precursor lesions were identified in the resected tubal tissue[95–99]. The detection of these lesions was greatly helped by protocols for complete histological examination of the ovaries and fallopian tubes, which focused attention on the fimbria (the finger-like projections at the distal end of the fallopian tube adjacent to the ovary) as the anatomical site of origin of many HGSCs[100,101].

The precursor lesion, STIC[102], and the FTE from which it is derived fulfil anatomical, phenotypic and biological criteria as the origin of most cases of HGSC. STICs have been reported in RRSO specimens from patients with germline *BRCA1* and *BRCA2* mutations[103] and in most advanced-stage HGSCs in the general population[104] (in which the fallopian tubes are intact for histological analysis). Two recent studies also indicate that the fimbria is the site of origin of incidentally discovered, sporadic HGSC (that is, in patients without a known genetic risk of ovarian cancer[105,106]). Phenotypically, HGSC and FTE share similar morphology, immunophenotype[107–112], gene expression patterns[113] and proteomic profile[114]. Biologically, HGSC, STIC and the 'p53-signature lesion' (a histologically normal but *TP53*-mutant fallopian tube lesion[101,102,115], which is the presumed precursor of STIC) have been shown to have evidence of DNA damage[115] and can share identical *TP53* mutations[115–120]. Human fallopian tube epithelial cells transformed *in vitro* generate tumours that recapitulate the morphology, immunophenotype and gene expression of human HGSC[121]. Finally, genetically engineered mouse models have demonstrated that mutations of *Trp53*, *Pten* and *Brca1* or *Brca2*, or *Trp53*, *Rb1* and *Brca1* in mouse tubal secretory cells result in STIC and HGSC[122] (K.R.C., unpublished results). The possibility that p53-signature lesions can develop into HGSC without an associated STIC intermediate needs to be explored.

Not all high-grade serous carcinomas start in the tube — the potential role of endosalpingiosis. It is unclear why certain sites of tubal epithelium are more susceptible to tumour initiation. Like the endometrium, which is present in the uterus and ectopically as endometriosis, tubal-type epithelium is similarly found in the fallopian tube and ectopically as endosalpingiosis[123]. Endosalpingiosis occurs in the ovary as microscopic or small macroscopic tubal-type cortical inclusion cysts (tubal CICs), on the tubal serosa as paratubal cysts and on other peritoneal surfaces. Within the eutopic fallopian tube, the basis for the strong predominance of STICs in the fimbria and not in the tubular portion is unclear. Recently, Auersperg[124] has demonstrated that epithelial cells in the OSE, fimbria and CICs are enriched for markers of stem cells (aldehyde dehydrogenase family 1 member A2 (ALDH1A2), homeobox protein NANOG, LIM homeobox protein 9 (LHX9) and secreted frizzled-related protein 1 (SFRP1)) and it has been proposed that the fimbria and OSE represent a developmental 'transition zone' that is prone to transformation[125]. The stem cell-like properties of the OSE were recently reviewed[126], and candidate fimbrial stem cells have also been identified[127,128]. The enrichment of stem cells and STICs in the fimbria may reflect a combination of potential developmental differences between fimbrial and tubular portions of the fallopian tube[129] and organ-extrinsic effects, such as the genotoxic effects of follicular fluid[130].

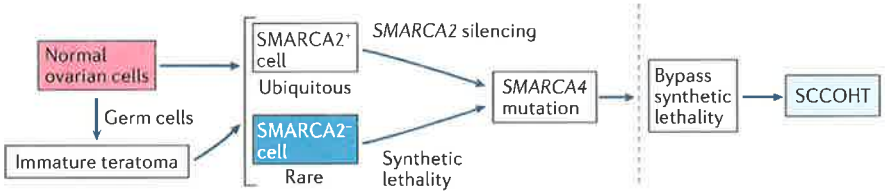

Figure 3 | **Pathogenesis of small cell carcinoma of the ovary, hypercalcaemic type.** Small cell carcinoma of the ovary, hypercalcaemic type (SCCOHT) is currently thought to develop either from a normal resident ovarian cell or from a cell within an immature teratoma[41]. SCCOHT almost universally lacks the two SWI/SNF ATPases: SMARCA4 (also known as BRG1) loss is caused by mutations and SMARCA2 (also known as BRM) loss is probably caused by epigenetic or post-transcriptional silencing. It is not known whether the absence of SMARCA2 expression in the tumours is due to silencing during tumorigenesis from a SMARCA2-positive (SMARCA2$^+$) cell of origin or because the tumours arise from a SMARCA2-negative (SMARCA2$^-$) cell of origin. Plausible explanations for the rarity of SCCOHT include a rare cell of origin — such as a SMARCA2$^-$ cell in the normal ovary[48] or within an immature teratoma[41] — or a biological barrier that makes it complicated to lose both SWI/SNF ATPases in response to synthetic lethality[49,50,159]. These possibilities are not mutually exclusive. For SCCOHT that develops in an immature teratoma, it is not clear whether it arises from the immature elements (for example, immature neuroepithelium), from a malignant component (for example, yolk sac tumour) or from one of the many normal (that is, mature) cell types.

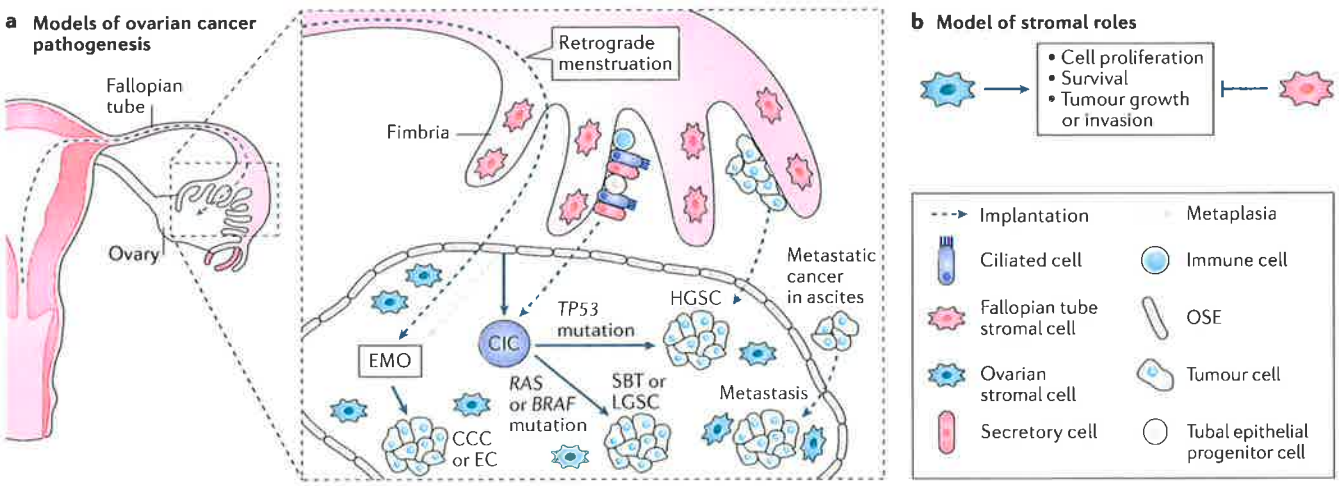

Figure 4 | **Microenvironments of the ovary versus fallopian tube in tumour biology.** The ability of primary ovarian carcinomas and metastatic carcinomas to grow as large masses in the ovary but not in the fallopian tube mucosa suggests a stimulatory role of ovarian stroma and inhibitory role of fallopian tube stroma in the progression of cancer precursor lesions and growth and/or invasion of cancer cells. Most high-grade serous carcinomas (HGSCs) arise from precursors in the fallopian tube fimbria that are called serous tubal intraepithelial carcinoma, which are thought to implant on the ovary and present as 'ovarian' HGSC, similar to metastatic carcinoma cells in ascites fluid (part **a**). Alternatively, some HGSCs may arise from intra-ovarian tubal epithelium, that is, ovarian endosalpingiosis, also called tubal-type cortical inclusion cysts (CICs). It is unclear whether CICs form by implantation of fimbrial epithelium or by metaplasia of ovarian surface epithelium (OSE). Larger tubal-type CICs are also thought to be an origin of serous borderline tumour (SBT) and low-grade serous carcinoma (LGSC). Ovarian endometriosis (EMO) is thought to arise by retrograde menstruation, by metaplasia or from Müllerian developmental remnants (not depicted). Although EMO is very common, it can acquire mutations and develop into clear cell carcinoma (CCC) or endometrioid carcinoma (EC). The propensity of all these tumours to grow as large ovarian masses rather than tubal masses supports the hypothesis that the ovarian stroma and fallopian tube stroma have stimulatory and inhibitory roles, respectively, in several aspects of tumorigenesis (part **b**). Dashed lines indicate processes or sources of cells (metaplasia or implantation from detached tubal cells, retrograde menstruation or ascites fluid). Solid lines indicate progression (EMO to CCC or EC; possible progression of tubal CIC to HGSC, SBT or LGSC).

Ovarian CICs are currently proposed as a possible origin of 'non-tubal' HGSC, that is, HGSC without evidence of fallopian tube mucosal involvement[131]. The epithelium lining of CICs can be tubal (secretory and ciliated), flat or OSE-type, or mixed, with appropriately corresponding immunophenotypes[108–112]. Although it is plausible that OSE-type CICs arise by invagination, CICs with tubal-type differentiation (endosalpingiosis) could develop by detachment and implantation of fimbrial epithelium, possibly containing a p53-signature lesion, through interruptions of the ovarian surface, or by metaplasia of the OSE following invagination into the cortex or by both processes. Current morphological and molecular evidence suggests that CICs may have neoplastic potential. Larger, mass-forming, tubal-type cystic lesions are thought to be a potential origin of low-grade serous tumours (serous borderline tumour and low-grade serous carcinoma)[132,133]. Although rare, STIC-like lesions or microscopic carcinoma have been identified in CICs[93,134], so it is plausible that CICs may be an origin of some HGSCs as well (that is, CIC–HGSC pathway; FIG. 4).

Endosalpingiosis can also be found in mouse ovaries. Lineage tracing experiments are currently being carried out to determine whether tubal-type CICs form by tubal epithelial detachment and implantation onto the ovary or by metaplasia of the OSE. In addition, we are investigating the neoplastic potential of these CICs in a genetically engineered mouse model of HGSC.

Salpingectomy and oral contraceptives as prevention strategies. Prophylactic (in the case of women with known increased risk of developing ovarian cancer) or opportunistic (that is, done at the time of other surgery or instead of tubal ligation) bilateral salpingectomy is a rational and cost-effective procedure designed to decrease ovarian cancer incidence by removing the fallopian tubes in women who have completed child bearing to eliminate the source (the fimbria) and transit mechanism (for endometriosis-related cancers) of the cancer precursor cells[62,135,136]. Nevertheless, studies on the long-term safety of salpingectomy, with respect to age of onset of menopause, are needed. Understanding the neoplastic potential of the various cells of origin (eutopic tubal epithelium versus endosalpingiosis) and how CICs form (tubal implantation versus metaplasia) is essential for understanding the mechanisms and effectiveness of this cancer prevention strategy. If CICs, the frequency of which increases with advancing age[108], form by tubal implantation, prophylactic salpingectomy will be most effective in younger patients. If CICs develop by metaplasia, bilateral salpingectomy may be a less effective prevention strategy, which has particular implications for patients with *BRCA1* and *BRCA2* mutations who opt for salpingectomy with delayed oophorectomy (to eliminate the cancer risk from the tube but delay surgical menopause in order to eliminate cancer risk from the ovary for as long as possible)[137–139].

Use of the oral contraceptive pill (OCP) is associated with significant duration-dependent decrease in ovarian cancer risk[61,140–145], including a variable protective effect in women with germline *BRCA1* or *BRCA2* mutations[146–148]. OCPs were historically thought to prevent ovarian cancer by interrupting ovulation[88], which induces stem cell marker expression in the OSE[149]. Although this may explain part of

the chemopreventive effect of OCPs, the identification of HGSC precursors in the fallopian tube has shifted research towards how OCPs affect the biology of eutopic or ectopic tubal secretory cells. OCPs may exert their chemopreventive effects by acting directly on tubal secretory cells (which express oestrogen and progesterone receptors), indirectly by preventing the monthly exposure of the tubal fimbria to the genotoxic follicular fluid that is released during ovulation[130,150] and/or by preventing damage-induced metaplasia of the OSE (if tubal-type CICs develop this way), thus providing a link between incessant ovulation and damage to FTE.

Regardless, it is essential to understand how OCPs regulate the biology of the tubal epithelium by studying their effects on the proliferation of mature tubal cells, tubal stem cells and cancer precursors (p53-signature lesions and STICs), and how they may regulate the immune cell repertoire, in both the eutopic tube and CICs. This will enhance our knowledge of the pathogenesis and prevention of ovarian cancer and perhaps help to identify other non-surgical prevention strategies for high-risk patients. Also, molecular markers that correlate with OCP-dependent reduced ovarian cancer risk could be used to predict whether new hormonal contraceptives will offer similar protection.

The ovarian microenvironment

Microenvironment of the normal ovary versus the fallopian tube. A wide variety of cancers that are not derived from normal ovarian cell types — primary ovarian carcinomas and metastatic cancers from the breast, lung and the gastrointestinal tract — can present as dominant ovarian masses. This highlights the long-observed but poorly understood concept that the ovary is a fertile and 'nurturing' environment for precancerous and cancerous lesions. The corollary to this is the presumably inhospitable microenvironment of the fallopian tube for growth of both primary tubal tumours, which present as ovarian disease, and metastases[151–153]. We hypothesize that a tumour-suppressive microenvironment in the fallopian tube may have evolved to protect against ectopic pregnancy, a condition that was lethal until the surgical era.

Several observations support the idea that cancer progression may be inhibited by the fallopian tube microenvironment but promoted by the ovarian microenvironment. As mentioned, HGSCs typically arise as a STIC but present as a dominant ovarian mass and not as a dominant tubal mass. Endometriosis may present with multifocal involvement of the pelvis, but it usually spares the fallopian tube mucosa. Cancers arising from endometriosis almost exclusively develop on the ovary, often in an endometriotic cyst. Finally, extra-ovarian tumours, many of which are likely to metastasize in ascites fluid, preferentially grow on the ovary and not on other sites in the abdomen, pelvis or the fallopian tube mucosa[152].

We have developed a simple model in which the microenvironments of the ovary, fallopian tube and peritoneum have distinct roles in tumour initiation, progression and/or growth (FIG. 4). It is plausible to propose a scenario in which ovarian cells elaborate factors that facilitate the implantation and initial growth and invasion of cancer cells (for HGSC or metastases to the ovary). The ability of these cancers to initiate (as STIC) or implant (HGSC and extra-ovarian metastases) on the fallopian tube mucosa but not grow as dominant masses suggests that the fallopian tube stroma, not the epithelium, is non-permissive or actively restricts tumour invasion and growth, at least compared with the ovary.

Microenvironment of the ovarian tumour versus normal ovary. Most ovarian tumours, both primary tumours and non-gynaecological metastases, are significantly larger than the normal ovary and among the largest cancers in the body. Therefore, almost all of the tumour stroma must be synthesized *de novo*. This raises important questions regarding the role of the stroma in cancer growth in the ovary. Do cancers grow as dominant ovarian masses because of a unique 'kick start' from the native ovary, or does the *de novo* intratumoural stroma continue to provide this stimulatory role, perhaps because it is still 'ovarian' in nature, or both? If the ovarian tumour stroma is indeed stimulatory, does this reflect its functional resemblance to the normal ovary?

Scully and Richardson[154] provided early evidence suggesting that ovarian tumour stroma possesses morphological and endocrinological properties of normal adult ovary, and noted that ovarian metastases from gastrointestinal tract carcinomas had stroma that was found neither in the primary gastrointestinal tract tumour nor in extra-ovarian metastases and contained luteinized, steroidogenic ovarian stromal cells. Several patients had either oestrogenic or androgenic manifestations that regressed after removal of the ovarian metastases. Similar histological findings can be observed in primary ovarian carcinomas[155].

Therefore, the stroma of some tumours in the ovary histologically resembles normal adult ovary, which may provide a stimulatory microenvironment for primary and metastatic tumours. A comparison of the transcriptional and proteomic profiles of ovarian tumour stroma with those of carcinomas in other organs (such as primary colon cancer versus ovarian metastases) could identify stimulatory factors that may explain the preferential growth of tumours in the ovary and could thereby be potential therapeutic targets.

Conclusions and future directions

Most patients with ovarian cancer present at an advanced clinical stage when curative therapy is no longer possible. Despite our best efforts at early detection[156], given the microscopic size of HGSC precursors in the fallopian tube and the free access of detached tubal cancer cells to the ovary and peritoneal cavity, we believe that primary prevention is the best way to decrease mortality. For an enigmatic and deadly cancer such as SCCOHT, it is essential to understand the cellular origin to prevent its genesis in girls with hereditary predisposition and to find new treatments for those individuals who develop the disease. For the most common biological precursor of ovarian cancer — endometriosis — it is important to identify the molecular determinants of its progression to cancer, to identify patients at risk and hopefully to identify new drug targets. For HGSC it is important to determine the effectiveness of opportunistic salpingectomy in decreasing ovarian cancer incidence and the neoplastic potential of ovarian endosalpingiosis, along with the mechanism by which it forms. In addition, it is important to understand how OCPs exert their protective effect, both to gain important insight into tubal stem cell biology and to identify effective biomarkers in order to maintain this beneficial 'side effect' of cancer prevention in future formulations of the pill and to determine whether it is translatable to increasingly popular long-acting reversible hormonal contraceptives (such as the Mirena levonorgestrel-releasing intrauterine system). New mouse models of HGSC and endometriosis-related cancers offer tractable models to complement studies in patients. Finally, studying the role that ovarian and tubal stromal cells have in cancer growth will hopefully reveal new biomarkers

and therapeutic opportunities. However, the development and implementation of biologically informed prevention strategies could be the most effective way to translate fundamental ovarian cancer research into improved patient outcome.

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Competing interests statement

The authors declare no competing interests.

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